

Null Results in Brief

Common Leptin Receptor Polymorphisms do not Modify the Effect of Alcohol Ingestion on Serum Leptin Levels in a Controlled Feeding and Alcohol Ingestion Study

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Abstract

We explored whether serum leptin response to alcohol ingestion was related to common leptin receptor gene polymorphisms, K109R (Lys¹⁰⁹Arg), Q223R (Gln²²³Arg), S343S [Ser(T)³⁴³Ser(C)], and K656N (Lys⁶⁵⁶Asn), of reported physiologic significance during a controlled intervention. Fifty-three participants rotated through three 8-week treatment periods and consumed 0, 15 (equivalent to one drink), or 30 g (equivalent to two drinks) of alcohol (95% ethanol in 12 ounces of orange juice) per day, in random order. During the controlled feeding periods, all food and beverages including alcoholic beverages were prepared and supplied by the staff of the Beltsville Human Nutrition Research Center's Human Study Facility (Beltsville, MD), and energy intake was adjusted to maintain a

constant weight. Blood was collected after an overnight fast on 3 separate days during the last week of each controlled feeding period and pooled for hormone analysis. Circulating serum leptin concentration was measured in duplicate by RIA and genotype analysis was done on DNA extracted from WBC using real-time PCR analysis amplification (TaqMan). Linear mixed models with a single random intercept reflecting a participant effect were used to estimate changes in serum leptin levels at 15 and 30 g of alcohol per day relative to 0 g of alcohol per day. No significant effects were found between common leptin receptor polymorphisms and serum leptin levels ($P \geq 0.26$). (Cancer Epidemiol Biomarkers Prev 2005;14(6):1576-8)

Introduction

Leptin is a hormone produced by the adipocyte *ob* gene, involved in energy balance, and may play a role in carcinogenesis and autoimmune disorders (1-15). We recently showed that moderate alcohol consumption (15-30 g of alcohol per day) increased serum leptin levels in postmenopausal women in a controlled feeding and alcohol ingestion study (1). In that study, 53 healthy, nonsmoking postmenopausal women completed a random-order, three-period crossover design in which each woman received zero (0 g of alcohol), one (15 g of alcohol), or two (30 g alcohol) drinks per day. After accounting for differences in body mass index, consumption of 15 or 30 g of alcohol per day increased serum leptin levels by 7.3% and 8.9%, respectively, over zero alcohol consumption, with younger women (i.e., 49-54 years); demonstrating a significantly stronger association of alcohol consumption level with the increase in serum leptin levels than older women (i.e., 55-79 years; 24.4% versus 3.7% for 30 g of alcohol per day relative to 0 g of alcohol, respectively).

To understand the potential mechanisms influencing serum leptin response to alcohol, we expanded our original findings to explore the leptin response to alcohol on common leptin receptor gene polymorphisms with reported

physiologic significance, K109R (Lys¹⁰⁹Arg), Q223R (Gln²²³Arg), S343S [Ser(T)³⁴³Ser(C)], and K656N (Lys⁶⁵⁶Asn; ref. 16). This is the first controlled feeding and alcohol ingestion study to examine variation in serum leptin response in relation to common polymorphisms of the leptin receptor.

Materials and Methods

A detailed description of the Women's Alcohol Study has been previously reported (17). Briefly, each participant rotated through three 8-week treatment periods and consumed 0, 15 (equivalent to one drink), or 30 g (equivalent to two drinks) of alcohol per day in random order. Alcohol was supplied to each participant as 95% ethanol (Everclear; Pharmco Products, Brookfield, CT) in orange juice (12 ounces) 1 to 2 hours before bedtime. Each controlled feeding period was preceded by a 2- to 5-week washout period during which time the participant consumed no alcohol. During the controlled feeding periods, all food and beverages were prepared and supplied by the Beltsville Human Nutrition Research Center's Human Study Facility (Beltsville, MD). Study participants were weighed each weekday at the Beltsville facility, and energy intake was adjusted to maintain a constant weight. Blood was collected after an overnight fast on 3 separate days during the last week of each controlled feeding period and pooled for hormone analysis. Circulating serum leptin concentration was measured in duplicate by RIA (Human Leptin RIA Kit, Linco Research, St. Charles, MO) and quantified using a Cobra Quantum Gamma Counter (Packard Instruments, Downers Grove, IL).

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Genotyping analysis was done on DNA extracted from WBC using a PureGene kit (Gentra Systems, Inc., Minneapolis, MN) and real-time PCR analysis amplification (TaqMan). Serum leptin concentrations were transformed to the natural log scale to make inferences on relative change and to make outcomes more normally distributed. Linear mixed models with a single random intercept reflecting a participant effect were used to estimate changes in serum leptin levels at 15 and 30 g of alcohol per day relative to 0 g of alcohol per day. Alcohol (three factors) by gene (two factors) interactions were examined by including appropriate cross product terms in the model. Adjustments for body mass index and period effects were done/done by including these terms as fixed effects in the linear mixed model.

Results

The majority of participants in this study were homozygous for the common allele at Lys¹⁰⁹Arg (66%), Ser³⁴³Ser (71%) and Lys⁶⁵⁶Asn (74%), but, for Gln²²³Arg, heterozygotes were the most common genotype (42%; Table 1). Due to the small number of individuals homozygous for the variant alleles, we examined the percentage of change in serum leptin concentration based upon a dichotomized genotype analysis which compared those homozygous for the common allele versus those heterozygous or homozygous for the variant allele.

After adjusting for body mass index and period effect, there were no significant differences in the percent change in serum leptin concentration with zero versus one or two drinks per day for these four common polymorphisms in the leptin receptor gene [$P \geq 0.26$, test of alcohol (three factors) by gene (two factors) based on a conditional F test; Table 2]. Power calculations were conducted based on an estimate of the within-subject variation obtained from the linear mixed model and performing a two-sample t test that compared change from zero to two drinks per day between homozygous common allele and heterozygous/homozygous variant allele groupings. The linear mixed model should provide increased power over this simple analysis. Assuming the distribution of Lys¹⁰⁹Arg alleles presented in Table 1 and an overall change of 8.9% (in geometric mean) as shown in Table 2, we were able to detect a difference between a 1% increase for the homozygous common allele and a 26% increase for the heterozygous/homozygous variant allele category with 80% power. The power calculations corresponding to the allele distribution for Ser³⁴³Ser and Lys⁶⁵⁶Asn were similar to those reported for Lys¹⁰⁹Arg. For Gln²²³Arg, where the allele distribution was very different from the other polymorphisms, we were able to detect a difference between a 5% decrease for the homozygous common allele and an 18% increase for the heterozygous/homozygous variant allele category with 80% power.

Table 1. Distribution of four common polymorphisms of the leptin receptor gene among participants of the women's alcohol study ($n = 53$)

| | Lys ¹⁰⁹ Arg <i>n</i> (%) | Gln ²²³ Arg | Ser ³⁴³ Ser | Lys ⁶⁵⁶ Asn |
|---------------------------|--|------------------------|------------------------|------------------------|
| Homozygous common allele | 33 (66%, AA) | 20 (38%, AA) | 37 (71%, TT) | 35 (74%, GG) |
| Heterozygous | 12 (24%, AG) | 22 (42%, AG) | 13 (25%, TC) | 12 (26%, GC) |
| Homozygous variant allele | 5 (10%, GG) | 10 (19%, GG) | 2 (4%, CC) | 0 (0%, CC) |
| Nondetectable | 3 | 1 | 1 | 6 |

Table 2. Percent change in serum leptin concentration with zero versus one or two drinks per day by four common polymorphisms (dichotomized genotype) in the leptin receptor gene ($n = 53$)

| | All | Lys ¹⁰⁹ Arg | Gln ²²³ Arg | Ser ³⁴³ Ser | Lys ⁶⁵⁶ Asn |
|--|---------|------------------------|------------------------|------------------------|------------------------|
| No. of drinks | 1 2 | 1 2 | 1 2 | 1 2 | 1 2 |
| All subjects | 7.3 8.9 | | | | |
| Homozygous common allele | | 10.2 11.4 | 3.9 7.5 | 4.1 4.8 | 11.0 13.5 |
| Heterozygous/homozygous variant allele | | 7.4 8.7 | 10.0 9.9 | 15.6 17.8 | 9.2 1.7 |
| Interaction test | | $P = 0.93$ | $P = 0.75$ | $P = 0.26$ | $P = 0.36$ |

NOTE: Test of alcohol (three factors) by gene (two factors) based on a conditional F test.

Discussion

Our recent finding that moderate alcohol consumption (15-30 g of alcohol per day) increased serum leptin levels is consistent with leptin's reported responsiveness to physiologic variation (18). This responsiveness may, in part, result from serum leptin binding to a membrane-associated leptin receptor with subsequent activation of the JAK-STAT signal transduction pathway and downstream STAT transcription factors, including several regulatory elements within the leptin promoter (18). The current study expanded our original findings to explore the leptin response to alcohol on common leptin receptor gene polymorphisms with reported physiologic significance. This includes increased adiposity (Arg²²³ variant; refs. 19-21), higher abdominal fat in postmenopausal women (Gln²²³Gln homozygotes or who carry the Asn⁶⁵⁶ allele; ref. 19), low body mass index and low systolic and diastolic blood pressure (Lys¹⁰⁹Arg; ref. 22), and variation in fat mass [Ser(Ty)³⁴³Ser(C) (ref. 23) and Lys⁶⁵⁶Asn (ref. 20)]. The results of this controlled intervention study found no significant modification of the serum leptin response to controlled alcohol ingestion by four common leptin receptor polymorphisms. The small number of participants limits the findings of this exploratory pilot study; however, adequate power exists for detecting a moderate (20-25%) difference between allelic categories. These results are further strengthened by the experimental design of the original intervention that maintained each participant's body mass index through control of caloric intake and food composition intake for >6 months and represent the first report of potential effects of leptin receptor polymorphisms on serum leptin levels following alcohol consumption in a controlled feeding study.

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